RIBOTYPING OF *CLOSTRIDIUM (C.) PERFRINGENS* ISOLATED FROM MINCED MEAT

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Background

C. perfringens is a Gram-positive, spore-formig, anaerobic rod. This bacterium belongs to the most important toxigenic microorganism causing food poisoning in man. *C. perfringens* could be detected in over 20 % of minced meat samples. Typing of these isolates is necessary for further information about origin and contamination routes of these microorganisms in slaughtering and cutting plants. As problems of typeability have been associated with many phenotypic techniques, numerous DNA-based methods have been developed. Ribotyping as DNA-based typing method has two major advantages: firstly due to the highly conserved genes encoding for rRNA one single probe can be used to type different species. And secondly, as most bacteria contain multiple ribosomal operons, number of visible DNA-fragments is suitable for intraspecies comparison. Suitability and repeatability of ribotyping for the characterization of *C. perfringens* has been demonstrated by Schalch et al. (1997).

Objective

Purpose of this study was to characterize a collection of *C. perfringens* isolated from minced meat beyond the species level in order to collect basic information for a contamination survey.

Materials and Methods

Strains. In this study 111 *C. perfringens* isolated from minced meat were investigated. Isolates had been stored in Cooked Meat Medium (Oxoid CM.,UK). They were purified and confirmed by Reverse CAMP test and Acid Phosphatase reagent (Schalch, 1996). Then they were stored on a Microbank™(Mast Diagnostica, Reinfeld, Germany) at -18 °C as described by Eisgruber et al. (1995).

DNA isolation. C. perfringens isolates were incubated under anaerobic conditions at + 37 °C for 16 h in Brain Heart Infusion Broth (Oxoid CM.UK) and then centrifuged (13 000 x g, 10 min). DNA was isolated with the guanidium thiocyanate method according to Pitcher et al. (1989) with the modifications described by Björkroth and Korkeala (1996).

Ribotyping. Ribotyping was carried out as described by Grimont and Grimont (1986). Following DNA isolation, the DNA concentrations were measured with a UV spectrophotometer (UV/VIS spectrometer Lambda 2; Perkin-Elmer, Norwalk, Conn.). Five micrograms of DNA was then digested with *Eco*RI at + 37 °C for maximum 2 hours. DIG-labeled phage lambda DNA cleaved with *Hind* III was used as a molecular weight marker (Boehringer Mannheim, Mannheim, Germany). DNA fragments were electrophoresed (19 h, 25 V) in 0.8 % agarose gel. The fragments were transferred from the gel to a nylon membrane by Southern Blot. After drying in room temperatur, the membrane was fixed at + 120 °C for 0.5 h. The membrane was weld in a plastic bag and prehybridized for 3 h in a 58 °C water bath. A cDNA probe was prepared from *Escherichia coli* 16S and 23S rRNA (Boehringer Mannheim) and labeled by incorparation of DIG-dUTP using avian myeloblastosis virus reverse transcriptase. The solutions used for hybridization, washes and detection of the DIG label were prepared according to DIG DNA labeling and detection kit (Boehringer Mannheim). Finally the membrane was dyed with nitrablue tetrazolium chloride (NBT) and X-phosphate, 4-toluidine salt (Boehringer Mannheim) overnight and washed with TE solution (10:1).

Results and discussion

Within 111 isolates, 109 distinctly different and two identical ribopatterns were detected. Figure 1 shows an example of the variability of ribopatterns. Also isolates from identical minced meat samples (n = 8) showed different ribopatterns in all cases.
The number of DIG labelled bands of *C. perfringens* isolates varied between 13 and 16. Typical ribopatterns of *C. perfringens* were as follows: 7 to 11 bands in between 23 kb and 4.3 kb, 0 to 4 bands in between 4.3 kb and 2.0 kb and 2

to 5 bands under 2.3 kb. The latter showed typical band patterns which could be differentiated easily. According to the band patterns smaller than 2.3 kb all 111*C. perfringens* isolates could be divided into 5 groups, named A to E. The profiles are presented in Figure 2. 70.3 % of all *C. perfringens* examinated belongs to profile typ A, 12.6 % to profile B and 9.9 % to profile C. Four *C. perfringens* strains showed different profile types (D, E).

Conclusion

In this study, 111 *C. perfringens* isolates from minced meat could be differentiated by ribotyping. This underlines the good ability to distinguish *C. perfringens* isolates by this method. Furthermore results clearly showed the great genetic variety of *C. perfringens* contaminating minced meat.

Literature

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Figure 1 **An example for ribotype patterns of *Clostridium perfringens* isolated from minced meat**

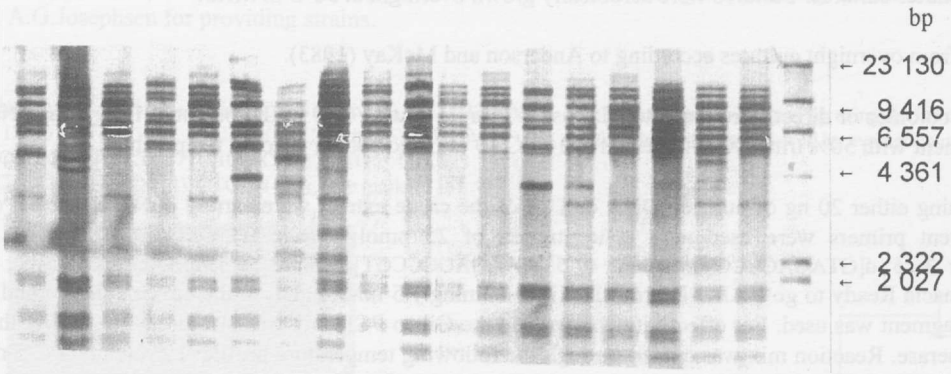

Figure 2 **Typical band patterns under 2322 bp of *Clostridium perfringens* isolates**